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Dietary weight loss-induced improvements in metabolic function are enhanced by exercise in people with obesity and prediabetes

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Abstract

The additional therapeutic effects of regular exercise during a dietary weight loss program in people with obesity and prediabetes are unclear. Here, we show whole-body (primarily muscle) insulin sensitivity (primary outcome) was two-fold greater (P=0.006) after 10% weight loss induced by calorie-restriction plus exercise training (Diet+EX; *n*=8, 6 women) than 10% weight loss induced by calorie restriction alone (Diet-ONLY; *n*=8, 4 women) in participants participating in two concurrent studies. The greater improvement in insulin sensitivity was accompanied by increased muscle expression of genes involved in mitochondrial biogenesis, energy metabolism and angiogenesis (secondary outcomes) in the Diet+EX group. There were no differences between groups in plasma branched-chain amino acids or markers of inflammation, and both interventions caused similar changes in the gut microbiome. Few adverse events were reported. These results demonstrate that regular exercise during a diet-induced weight loss program has profound additional metabolic benefits in people with obesity and prediabetes.

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AUTHOR CONTRIBUTIONS

JWB, BDK, GIS, GR, JY, RK, and BWP performed sample and data analyses. JWB, BDK, GIS, GGS, KK, and MLK conducted the clinical studies. GIS and SK designed the study. JWB, BDK, GIS, BWP, RK and SK interpreted the data and wrote the manuscript. All authors critically reviewed and edited the manuscript.

COMPETING INTERESTS STATEMENT

S.K. serves on scientific advisory boards for Altimmune and Merck. All other authors declare they have no competing interests.

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Insulin-resistant glucose metabolism is the most common metabolic abnormality associated with obesity and is involved in the pathogenesis of obesity-related cardiometabolic co-morbidities, including dyslipidemia, nonalcoholic fatty liver disease (NAFLD), type 2 diabetes and cardiovascular disease [1]. Diet-induced weight loss and exercise training without weight loss improve insulin sensitivity and the metabolic abnormalities associated with obesity [2–6]. Therefore, lifestyle interventions that combine an energy-deficit diet and increased physical activity are recommended as the primary therapy for people with obesity [7–9]. However, the additional benefit of exercise training, when provided in conjunction with diet-induced weight loss, on insulin sensitivity and metabolic health is unclear because of conflicting data from previous studies, which have reported an additive effect [10–14] or no additional effect of exercise training on insulin sensitivity [15–19]. The reason(s) for the discrepant findings among studies could be related to differences in the methods used to assess insulin sensitivity or the duration between the most recent exercise bout and metabolic testing. A better understanding of the potential additional therapeutic effects of exercise training beyond diet-induced weight loss alone is important because exercise programs are often more difficult to implement than calorie restriction in people with obesity due to increased barriers to exercise in this population [20].

The main purpose of the present study was to test the hypothesis that moderate 10% diet-induced weight loss in conjunction with multi-modal exercise training causes greater improvement in whole-body (primarily muscle [21]) insulin sensitivity (primary outcome) than matched 10% weight loss induced by calorie restriction alone in people with obesity and prediabetes. In addition, we assessed several secondary outcomes to provide a better understanding of whether weight loss with exercise training affects a constellation of other key therapeutic cardiometabolic outcomes effects and specific putative mechanisms proposed to regulate insulin action compared with weight loss alone. To this end, we conducted a comprehensive evaluation of the following outcomes in people with obesity and prediabetes before and after 10% weight loss induced within ~5 months by either calorie restriction alone (Diet-ONLY) or calorie restriction plus supervised exercise training (Diet+EX): i) whole-body (muscle) and liver insulin sensitivity, assessed by using the hyperinsulinemic-euglycemic clamp procedure in conjunction with stable isotopically labelled glucose tracer infusion; ii) β-cell function and insulin kinetics; iii) metabolic response to glucose ingestion assessed for 3 hours and ingestion of multiple mixed-meals assessed over 24 hours; iv) cardiometabolic risk factors (plasma lipid profile, markers of inflammation, plasma branched-chain amino acid (BCAA) concentrations, body composition (fat-free mass, fat mass and intrahepatic triglyceride [IHTG] content), cardiorespiratory fitness and muscle strength); v) skeletal muscle tissue global transcriptomics; vi) the plasma proteome; and vii) the composition of the gut microbiome. The assessment of the secondary outcomes was considered “hypothesis generating” so the statistical analysis of differences between groups was not corrected for multiple testing. The intervention in the Diet-ONLY group involved weekly individual dietary and behavioral education sessions with all food provided as packed-out meals to enhance compliance and minimize the potential confounding effect of differences in macronutrient intake between groups on our study outcomes. The Diet+EX group had the same dietary intervention as the Diet-ONLY group plus 6 hours of exercise training

per week (four 1-hour supervised exercise sessions and two 1-hour sessions performed at home). A plant-forward diet, which is low in fat, sodium, and refined carbohydrates, and high in complex carbohydrates, was used as the dietary intervention because this diet enhances weight loss and has considerable beneficial effects on glycemic control and other cardiometabolic abnormalities in people with obesity [22–24].

RESULTS

Muscle strength and cardiorespiratory fitness

Mean weight loss was nearly identical in both groups (10.4 ± 0.9% and 10.6 ± 1.1% weight loss in the Diet-ONLY and Diet+EX groups, respectively), and was associated with decreases in body fat, fat-free mass (FFM), appendicular lean mass and IHTG content that were not different between groups (Table 1). The duration of the intervention in the Diet-ONLY group (20.6 ± 1.8 weeks) was not different than the duration of the intervention in the Diet+EX group (18.2 ± 1.3 weeks) (P=0.31). Participants in the Diet+EX group attended 97 ± 3% of the supervised exercise sessions. Muscle strength, assessed as the total weight (in kg) lifted during leg press, knee flexion, seated row, and chest press one-repetition maximal (1-RM) strength tests, increased more in the Diet+EX group (13 ± 4% change from baseline) than in the Diet-ONLY group (−2 ± 3% change from baseline) (P=0.025) (Table 1 and Extended data Table 1). In addition, cardiorespiratory fitness, assessed as peak oxygen consumption (VO_{2peak}) during incremental treadmill exercise to volitional exhaustion, increased more in the Diet+EX group than the Diet-ONLY group when the data were expressed as the total rate of oxygen consumption (L/min) (+10 ± 4% vs −6 ± 1%, respectively; P=0.007), the rate of oxygen consumption in relation to FFM (mL/kg FFM/min) (+13 ± 4% vs −3 ± 1%, respectively; P=0.005), and the rate of oxygen consumption in relation to body weight (mL/kg/min) (+23 ± 4% vs +6 ± 1%, respectively; P=0.004) (Table 1 and Extended data Table 1).

Hemoglobin A1c (HbA1c), fasting plasma glucose triglycerides, BCAA, HDL-cholesterol and total cholesterol and PAI-1 concentrations decreased, or tended to decrease after weight loss in both groups, without a difference between groups (Table 1 and Extended Data Table 2). Fasting plasma insulin decreased more after weight loss in Diet+EX group than in the Diet-ONLY group (P=0.009) (Table 1). Fasting plasma NEFA concentrations were unchanged in the Diet-ONLY group but increased by ~50% in the Diet+EX group (difference between groups, P=0.022). Weight loss did not change plasma LDL-cholesterol, C-reactive protein concentration or concentrations of many proinflammatory (MCP-1, IL-6, TNF-α, IL-17, IL-1β, IFN-γ) or anti-inflammatory (IL-10) cytokines (Extended Data Table 2).

Metabolic response to glucose and mixed-meal ingestion

Plasma glucose concentrations for 3 hours after participants ingested 75 g of glucose and for 24 hours with the ingestion of three mixed-meals (each providing one-third of the participant's estimated energy requirement [25] given at 0700h [t=0 hours], 1300h [t=6 hours] and 1900h [t=12 hours] Extended Data Figure 1) decreased after weight loss in both groups, without a difference in the decline in plasma glucose area under the curves (AUCs) between

groups (Fig. 1A and 2A and Extended Data Table 3). Plasma insulin concentration AUCs for 3 hours after glucose ingestion and for 24 hours (with 3 mixed meals) decreased after weight loss in both groups, but the decreases were greater in the Diet+EX than in the Diet-ONLY group, P=0.008 and P=0.030, respectively (Fig. 1B and 2B and Extended Data Table 3). The decrease in postprandial insulin concentration in the Diet-ONLY group was due to a decrease in insulin secretion rate (ISR), whereas the greater decrease in postprandial insulin in the Diet+EX group was due to both a decrease in ISR and an increase in insulin clearance rate (ICR) (Fig. 1C, 1D, 2C and 2D and Extended Data Table 3). β-cell function, assessed as the relationship between ISR and plasma glucose concentration during the first 30 minutes after glucose ingestion when plasma glucose concentration is rapidly rising, was not different after than before weight loss in either the Diet+EX or the Diet-ONLY groups (Fig. 3A). In contrast, ICR at any given plasma insulin concentration was greater after than before weight loss in the Diet+EX group but did not change in the Diet-ONLY group (Fig. 3B). Plasma NEFA 24-h AUC decreased after weight loss in Diet-ONLY group but increased after weight loss in the Diet+EX group, so the change in NEFA 24-h AUC was significantly different between groups (P=0.003) (Fig. 2E and Extended Data Table 3).

Insulin sensitivity

Whole-body insulin sensitivity (assessed as the glucose disposal rate per kg FFM divided by plasma insulin concentration during a hyperinsulinemic-euglycemic clamp procedure, which primarily represents skeletal muscle insulin sensitivity [21]) increased after weight loss in both groups, but the increase was ~3-fold greater in the Diet+EX than the Diet-ONLY group (P=0.006) (Fig. 4A and Extended Data Table 3). Hepatic insulin sensitivity (assessed as the reciprocal of the product of basal endogenous glucose production rate and basal plasma insulin concentration [26]) increased after weight loss in both groups, but the increase was ~2-fold greater in the Diet+EX than the Diet-ONLY group (P=0.014) (Fig. 4B and Extended Data Table 3). The Matsuda insulin sensitivity index (assessed by using plasma glucose and insulin concentrations immediately before and for two hours after glucose ingestion [27]) increased after weight loss in both groups, but the increase was ~4-fold greater in the Diet+EX than the Diet-ONLY group (P=0.037) (Fig. 4C and Extended Data Table 3). The homeostasis model assessment of insulin resistance (HOMA-IR) values (assessed by using fasting plasma glucose and insulin concentrations) decreased after weight loss in both the Diet-ONLY and Diet+EX groups, but the decrease was greater in the Diet+EX than the Diet-ONLY group (P=0.001) (Table 1).

Skeletal muscle transcriptome

RNA-sequencing was used to evaluate global changes in gene expression in the vastus lateralis muscle before and after weight loss. There were 877 differentially expressed genes (DEGs) (623 upregulated and 254 downregulated) in the Diet+EX group and only one DEG (heme oxygenase 1; *HMOX1*), which was downregulated in the Diet-ONLY group (Fig. 5A and Supplementary Table 1). The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to assess enrichment for Gene Ontology Biological Processes (GO-BP) among the DEGs in the Diet+EX group. DEGs were highly enriched in 20 pathways (18 upregulated and 2 downregulated). Upregulated DEGs were predominantly enriched in many gene sets regulating mitochondrial energy metabolism

and angiogenesis (Fig. 5B). Consistent with the pathway analysis, the expression of specific genes involved in mitochondrial biogenesis and energy metabolism (peroxisome proliferator-activated receptor γ coactivator 1 α, *PPARGC1A*; mitochondrial fission factor, *MFF*; citrate synthase, *CS*; malate dehydrogenase 1, *MDH1*; cytochrome C somatic, *CYCS*; ATP Synthase F1 Subunit α, *ATP5F1A*; acyl-CoA dehydrogenase very long chain, *ACADVL*; acyl-CoA dehydrogenase medium chain, *ACADM*; lipoprotein lipase, *LPL*; and cluster of differentiation 36, *CD36*) and angiogenesis (vascular endothelial growth factor α, *VEGFA*; and vascular endothelial growth factor receptor 2, *KDR*) increased more in the Diet+EX group than the Diet-ONLY group (All $P<0.05$) (Fig. 5C and Fig. 5D and Extended Data Table 4). The downregulated DEGs in the Diet+EX group were enriched in the following two pathways: “Positive regulation of osteoblast differentiation” and “Skeletal muscle contraction”.

Plasma proteome

A total of 4,684 plasma proteins were identified before and after weight loss by using the aptamer-based SOMAscan proteomics assay (Somalogic, Boulder, CO) to assess whether: i) the circulating proteome was altered by weight loss with and without concomitant exercise; and ii) alterations in the skeletal muscle gene transcriptome were associated with a corresponding change in the encoded circulating proteins. There were 111 (50 upregulated and 61 downregulated) and 164 (77 upregulated and 87 downregulated) proteins with differential abundance after weight loss in the Diet-ONLY and Diet+EX groups, respectively (Fig. 6A). By using the protein enrichment analysis of the GO-BP, we found that the most enriched pathways were upregulated in the Diet+EX group but were downregulated in the Diet-ONLY group (Fig. 6B). GO-BP processes related to autophagy and ribose and purine biosynthesis were the most significantly upregulated pathways in the Diet+EX group, whereas these pathways were downregulated in the Diet-ONLY group (Fig. 6B). We then analyzed Gene Ontology Cellular Components (GO-CC) categories to better understand the source of differentially abundant proteins in plasma. The most significantly enriched cellular component in the Diet+EX group was an upregulation of “Mitochondrion”, which was markedly downregulated in the Diet-ONLY group. The cellular component “Mitochondrial matrix” was also downregulated in the Diet-ONLY group and upregulated in the Diet+EX group. In addition, 12 circulating proteins involved in mitochondrial biology and energy metabolism and the genes in muscle encoding those proteins were increased in the Diet+EX group (Fig. 6C), but not in the Diet-ONLY group. These data suggest the changes in skeletal muscle gene expression induced by Diet+EX produce several proteins that are released into the circulation and increase their plasma concentrations.

Gut microbiome

To assess the effect of the two interventions on the composition of the gut microbiome, we used 16S rRNA amplicon sequencing to assess the microbiota in fecal samples collected before (T0), during (T1 and T2, which were obtained at about 1 month and 2 months after starting the intervention when participants had lost 5.2 ± 0.7 and 8.6 ± 0.8% body weight, respectively), and while weight-stable at the end of the intervention (T3, which were obtained at about 4–5 months after starting the intervention). Alpha diversity was unchanged and beta diversity changed in the same direction and to a similar magnitude across T0, T1,

T2 and T3 in both the Diet-ONLY and Diet+EX groups (Extended Data Fig. 2), so data were analyzed separately and with both treatment groups combined to enhance the ability to identify diet and weight loss-induced changes in the gut microbiome.

Alpha diversity, measured as either Chao richness or Shannon Entropy of the amplicon sequencing variants (ASVs), was not different at any timepoint during the intervention than the values at baseline in the combined analysis (Fig. 7A), or in either group (Extended Data Fig. 2A). There was an overall effect of time on beta diversity, assessed by distance-based Redundancy Analysis (ordination plot) and Adonis through permutational multivariate analysis of variance (PERMANOVA; $P<0.05$) of weighted UniFrac distances (Fig. 7B and Extended Data Fig. 2B). Gut microbial composition changed from T0 to T1 of the intervention (PERMANOVA $P<0.05$), without further changes at T2 and T3 despite continued weight loss (PERMANOVA $P>0.05$ for each pairwise comparisons among T1, T2, and T3), demonstrating most of the change in the composition of the gut microbiome occurred within the first month after beginning the intervention in both the combined and by group analyses (Fig. 7B and Extended Data Fig. 2B). In addition, community changes over time were not different between the Diet+EX and the Diet-ONLY (group x timepoint interaction using PERMANOVA: $P = 0.458$, Extended Data Fig. 2B).

Changes in ASVs were analyzed by using linear mixed effects negative binomial model analysis comparing ASVs at T1 compared to T0, which coincided with the largest difference in beta diversity between time points (Supplementary Tables 2 and 3). The 10 ASVs with the largest increase and decrease from T0 to T1 were identified (Fig. 7C). The log-ratio [28] of the ASVs that changed from T0 to T1 remained greater than T0 at T2 and T3 in the pooled analysis (Fig. 7C) with no difference in the log-ratio of these ASVs between the Diet+EX and Diet-ONLY groups (p=0.603 by linear mixed effects model) (Extended Data Fig. 2C).

Adverse events

There were very few study-related adverse events in either group. One participant in the Diet-ONLY group experienced anxiety during baseline MRI scanning. In the Diet+EX group, one participant experienced anxiety during baseline MRI scanning, one had a syncopal episode after the muscle biopsy procedure, one reported a headache of unknown cause and one reported significant muscle soreness after a bout of exercise the previous day.

DISCUSSION

The purpose of the present study was to assess the potential clinical and metabolic importance of incorporating multi-modal exercise training as part of a weight loss therapy program in people with obesity and prediabetes. Accordingly, we evaluated the effect of 10% diet-induced weight loss with and without concomitant multi-modal exercise training on: i) whole-body [muscle] insulin sensitivity assessed by using the hyperinsulinemic clamp procedure (primary outcome); ii) cardiometabolic secondary outcomes (hepatic insulin sensitivity, body composition, cardiorespiratory fitness, muscle strength, metabolic responses to glucose ingestion and repeated mixed-meal consumption, β-cell function and insulin kinetics); and iii) putative mechanisms responsible for weight loss and exercise-induced improvements in insulin action, namely skeletal muscle transcriptomics, plasma

proteomics, circulating markers of inflammation, plasma BCAA and NEFA concentrations, and the gut microbiome. Our data demonstrate that 10% weight loss induced by diet alone or diet plus exercise training improved multi-organ insulin sensitivity and decreased basal and postprandial insulin concentrations. However, we found the increase in whole-body insulin sensitivity was much greater after weight loss induced by diet and exercise than matched weight loss induced by diet alone. We also found that changes in our secondary outcomes, namely the increase in hepatic insulin sensitivity and the decreases in basal, postprandial and 24-hour plasma insulin concentrations, were greater after weight loss induced by diet and exercise than matched weight loss induced by diet alone. However, the conclusions from these secondary outcomes should be considered "hypothesis generating" and the statistical analysis of differences between groups was not corrected for multiple testing. An unbiased assessment of skeletal muscle transcriptomics and the plasma proteome suggest weight loss induced by diet plus exercise caused marked remodeling of the mitochondrial and vascular network of skeletal muscle by increasing mitochondrial biogenesis, mitochondrial content and angiogenesis. Together, these data demonstrate that multi-modal exercise has profound additional beneficial effects on multi-organ insulin sensitivity and insulin kinetics, beyond weight loss alone, in people with obesity and prediabetes. These results underscore the importance of including exercise training as a component of obesity management, in addition to a calorie deficit diet, even though exercise *per se* typically has minimal effects on body weight.

The timing of testing in relation to the last bout of exercise has an important influence on the assessment of insulin sensitivity. Even a single 45-minute session of endurance exercise improves insulin sensitivity measured 48 hours later and additional short-term (6 weeks) exercise training in the same participants caused a further increase in insulin sensitivity [3]. However, the increase in insulin sensitivity after an acute bout of exercise or exercise training decreases over time and is usually back to baseline within 72 to 96 hours [29,30]. Therefore, evaluating metabolic function several days after the last bout of exercise could underestimate the beneficial effect of an exercise training program on insulin action. In the present study, we used the hyperinsulinemic-euglycemic clamp procedure, in conjunction with stable isotopically labeled glucose tracer infusion, to provide a robust assessment of hepatic and whole-body (muscle) insulin sensitivity at ~17.5 and ~21 hours after the last bout of exercise, respectively. Our data demonstrate that regular exercise training has considerable additive effects in improving insulin sensitivity compared with weight loss alone, which is likely due to both the chronic and acute effects of exercise.

The mechanism responsible for the additive effect of exercise on insulin-stimulated glucose uptake is likely related to exercise-induced changes in skeletal muscle biology. We found skeletal muscle expression of genes involved in mitochondrial energy metabolism and substrate oxidation increased more in the Diet+EX group than the Diet-ONLY group, which is consistent with the results from previous studies [31,32]. Moreover, an analysis of the plasma several circulating proteins involved in skeletal muscle energy metabolism and mitochondrial biology after weight loss in the Diet+EX compared with the Diet-ONLY group. It has been proposed that decreased skeletal muscle mitochondrial content and function are involved in the proteome reinforced the skeletal muscle transcriptomic results by demonstrating an increase in pathogenesis of muscle insulin resistance [33,34], which

suggests that the increase in muscle mitochondrial content or function in the Diet+EX group could have contributed to the greater increase in muscle insulin sensitivity compared to the Diet-ONLY group. It is also likely that the potent effects of exercise on insulin sensitivity were mediated by additional factors that increase skeletal muscle glucose delivery, uptake and metabolism, such as exercise-induced increases in muscle capillary density and capillary:fiber ratio [35], tissue perfusion [36], the number of plasma membrane GLUT4 glucose transporters [37], and insulin-stimulated muscle glycogen synthesis [3].

We evaluated the effect of matched weight loss with and without exercise training on specific circulating factors that are purportedly involved in the pathogenesis of insulin resistance, namely plasma BCAAs, cytokines, and NEFAs [38]. Weight loss decreased fasting plasma BCAA concentrations in both the Diet+EX and Diet-ONLY groups, without a difference in the magnitude of the reduction in plasma BCAAs between groups. Fasting plasma cytokine concentrations were not affected by weight loss in the Diet-ONLY and Diet+EX groups apart from plasma PAI-1, which decreased by ~55% in both groups after weight loss. We recently found that plasma PAI-1 concentrations are inversely associated with hepatic and skeletal muscle insulin sensitivity [39], supporting the potential importance of PAI-1 in regulating insulin sensitivity. Plasma 24-hour NEFA concentration AUC decreased after weight loss in the Diet-ONLY group, but increased after weight loss in the Diet+EX group, presumably because of exercise-induced stimulation of adipose tissue lipolytic activity [40]. These findings suggest that reductions in plasma BCAA and PAI-1 concentration contribute to weight loss-induced improvements in insulin sensitivity, but that plasma BCAAs, cytokines and NEFAs do not explain the greater improvement in insulin sensitivity observed after moderate weight loss with exercise training than moderate weight loss alone.

Weight loss with or without exercise training did not affect β-cell function, assessed as the ISR in relation to plasma glucose concentration during the first 30 minutes after glucose ingestion. Nonetheless, insulin secretion rates after glucose ingestion or during 24 hours with mixed-meal consumption were lower after than before weight loss in both groups, due to a decreased stimulus for insulin secretion caused by lower postprandial plasma glucose concentrations. Although basal, postprandial, and 24-h plasma insulin concentrations decreased after weight loss in both groups, the decrease was greater in the Diet+EX than the Diet-ONLY group. The difference in plasma insulin between groups was related to an increase in ICR after weight loss in the Diet+EX group, which did not change after weight loss in the Diet-ONLY group. The mechanism responsible for the increase in ICR in the Diet+EX group is likely related to exercise training-induced increases in insulin extraction by the liver [41,42] and skeletal muscle [43]. Together, these data demonstrate that weight loss with or without exercise does not affect β-cell function in people with obesity and prediabetes, and that diet-induced weight loss decreases plasma insulin concentrations by decreasing ISR, whereas diet-induced weight loss plus exercise training decreases plasma insulin concentrations by decreasing ISR and increasing ICR.

Distinct alterations in the composition of the gut microbiome are associated with obesity, the metabolic syndrome, and type 2 diabetes [44]. Moreover, transplantation of fecal microbiota from lean healthy donors to people with obesity and metabolic syndrome improves insulin

sensitivity in the recipients, suggesting the composition of the gut microbiome can directly affect metabolic function [45,46]. Therefore, we evaluated fecal samples obtained at baseline, at ~1 and ~2 months during the weight loss intervention, and at ~4–5 months, after participants lost about 10% of their body weight and were weight stable for several weeks. Our data demonstrate that consumption of a plant-forward diet causes marked changes in the composition of the gut microbiome beta diversity within 1 month of initiating diet therapy, without further changes thereafter, despite continued weight loss. Moreover, the change in microbial diversity was not different between the Diet-ONLY and Diet+EX groups. These results demonstrate the profound effect of dietary composition on beta diversity that is not further modified by regular exercise. The changes in the global microbial communities were reflected by specific microbial taxa that responded to the dietary intervention after ~1 month, a change which persisted with continued intervention in both groups. However, we are unable to determine whether these alterations in the gut microbiome contributed to the improvements in insulin sensitivity observed in our participants or were simply associated with the change in dietary composition without influencing insulin action.

Our study has some limitations. First, the participants in the Diet+EX group completed all post-intervention metabolic studies the day after completing an exercise bout, so we cannot determine the separate contributions from chronic and recent exercise to the greater increase in insulin sensitivity observed in the Diet+EX than in the Diet-ONLY group. Nonetheless, our study design represents an assessment of the effect of regular exercise and avoids the confounding effect of detraining on insulin sensitivity. Second, our study was conducted in people with obesity and prediabetes who participated in a short-term (~5-months) supervised multi-modal exercise training program and who were given a plant-forward diet because this type of diet enhances weight loss and has beneficial effects on cardiometabolic outcomes. [22–24] Therefore, we do not know if similar results would be obtained in other patient populations, or after treatment with different types of dietary and exercise training regimens. Third, the intensity of the lifestyle intervention in our study (all meals provided and supervised exercise training), which was needed to reliably test our hypothesis, is not feasible for most people in the “real world.” Fourth our study was not a randomized trial. However, to help ensure balance between the Diet-ONLY and Diet+EX groups, the Diet-ONLY and Diet+EX groups were studied simultaneously to avoid the limitations of historical controls and the eligibility and exclusion criteria were identical for both the Diet-ONLY and Diet+EX groups. Accordingly, the key baseline characteristics were similar in the Diet-ONLY and Diet+EX groups. Finally, our study included many secondary outcomes that should be considered “hypothesis generating” and the statistical analysis of differences between groups was not corrected for multiple testing. However, the secondary outcomes that improved more in the Diet+EX than the Diet-ONLY group, such as cardiorespiratory fitness, muscle strength, and muscle expression of genes related to mitochondria and energy metabolism, are consistent with the expected effects of exercise training despite the increased chance of a type 1 statistical error.

In summary, the results from the present study demonstrate that multi-modal exercise, conducted in conjunction with diet-induced weight loss in people with obesity and prediabetes, caused greater improvement in whole-body (muscle) insulin sensitivity and in key secondary outcomes, including hepatic insulin sensitivity, cardiorespiratory fitness

and muscle strength, than matched weight loss achieved solely by calorie restriction. These findings underscore the importance of targeting skeletal muscle biology in regulating metabolic health and physical function. Accordingly, exercise training should be incorporated as part of any weight loss program to maximize the cardiometabolic benefits of lifestyle therapy.

METHODS

Participants

The overall study protocol is shown in Extended Data Fig. 1. Twenty-one adults with obesity and prediabetes participated in one of two interventions: i) 10% weight loss induced by calorie restriction alone (Diet-ONLY; n=11, 8 self-reported as females at birth); or ii) 10% weight loss induced by calorie restriction plus exercise training (Diet+EX; n=10, 7 self-reported as females at birth). Subjects provided written, informed consent before participating in this study, which was approved by the Institutional Review Board within the Human Research Protection Office at Washington University School of Medicine in St. Louis, MO and registered in ClinicalTrials.gov (NCT02706262 and NCT02706288). All subjects were reimbursed for time and effort. Participants in the Diet-ONLY arm were also part of a larger ongoing clinical trial comparing the metabolic effects of 10% weight loss induced by three different dietary interventions without exercise training (NCT02706262). The participants in each group were enrolled in their respective trials concurrently between April 2016 and November 2018. The Diet-ONLY and Diet+EX groups were studied simultaneously to avoid the limitations of historical controls and the eligibility and exclusion criteria were identical for both groups. All participants were recruited by using the Volunteers for Health database at Washington University School of Medicine and by local postings between April 2016 and November 2018. Study visits were conducted in the Clinical Translational Research Unit (CTRU) and the Center for Clinical Imaging Research at Washington University School of Medicine in St. Louis, MO. All subjects completed a comprehensive screening evaluation that included a medical history and physical examination, standard blood tests, hemoglobin A1c (HbA1c), an oral glucose tolerance test (OGTT) to determine eligibility. The following inclusion criteria were required: BMI 30.0–49.9 kg/m^2, prediabetes (HbA1c ≥5.7–6.4% or fasting plasma glucose concentration 100–125 mg/dL, or 2-hr OGTT plasma glucose concentration 140–199 mg/dL). In addition, all participants had evidence of whole-body insulin resistance, defined as a glucose infusion rate <8 mg/kg FFM/min during a hyperinsulinemic-euglycemic clamp procedure [47]. Potential participants who had a history of diabetes, liver disease other than NAFLD, were taking medications that could affect the study outcomes, consumed excessive amounts of alcohol (>21 units of alcohol per week for men and >14 units of alcohol per week for women), or were pregnant or lactating were excluded. A total of 16 subjects completed baseline and post-weight loss testing procedures and were included in the comparison of the Diet-ONLY (n=8, 4 females; mean age 43 ± 3 years old) and Diet+EX (n=8, 6 females; mean age 46 ± 3 years old) groups (Extended Data Fig. 3). Data collection was not performed blind to the conditions of the experiments.

Body composition analyses

Body fat mass, FFM and appendicular lean mass were determined by using dual-energy X-ray absorptiometry (DXA, Lunar iDXA, GE Healthcare Lunar, Madison, WI). Intrahepatic triglyceride (IHTG) content was determined by using magnetic resonance imaging (3-T superconducting magnet; Siemens, Iselin, NJ) [48]. One participant in the Diet+EX group was unable to complete the MRI scans due to claustrophobia.

Cardiorespiratory fitness and maximal strength assessment

Cardiorespiratory fitness (peak oxygen consumption, VO_{2peak}) was determined by using indirect calorimetry (ParvoMedics, Sandy, UT) during a progressive treadmill test, in which speed and grade were adjusted in two-minute stages until volitional exhaustion. Maximal effort during the test was defined as achieving at least two of the following criteria: 1) a respiratory exchange ratio at maximal exercise >1.15, 2) a leveling off of heart rate with no more than a 3 beat/min increase between the final two workloads, and 3) plateau in oxygen consumption with <0.2 L/min increase between the final two workloads. On a separate occasion, participants attended an orientation session to become familiar with the weight-lifting equipment (Hoist multi-station weight machine, Hoist Fitness Systems, Poway, CA) and to receive instruction on correct technique for the following exercises (all bilateral): leg press, knee flexion, chest press, and seated row. Subjects returned to the research unit ~1-wk later to determine maximal strength (one-repetition maximum, 1-RM) for each of these exercises [50]. For technical reasons, data were not obtained for cardiorespiratory fitness and maximal strength from 1 and 2 participants, respectively, in the Diet-ONLY group.

3-h oral glucose tolerance test

Subjects were admitted to the outpatient unit of the CTRU at Washington University School of Medicine at 0700 hours after subjects fasted for approximately 11 hours overnight at home. An intravenous catheter was inserted into an antecubital or hand vein for serial blood sampling. Plasma glucose, insulin, and C-peptide concentrations were determined 15, 10, and 5 minutes before and 10, 20, 30, 60, 90, 120, 150, and 180 minutes after consuming a 75-g glucose beverage. The average of the three baseline samples obtained at 15, 10, and 5 minutes before consuming the 75-g glucose beverage was used as the 0-minute value for glucose, insulin, and plasma C-peptide concentrations. Due to technical issues, one participant in the Diet-ONLY group did not complete the oral glucose tolerance test after weight loss.

24-h blood sampling and assessment of insulin sensitivity

The timeline for the inpatient studies is shown in Extended Data Fig. 1d. Participants were admitted to the CTRU at 1800 h for ~48 hours and consumed a standard meal containing one-third of their estimated energy requirements [25] at 1900 h. At 0630 h on the morning of day 2, a catheter was inserted into an antecubital vein for 24-h serial blood sampling. Blood samples were obtained hourly from 0700 h to 2300 h on day 2 and from 0500 h to 0700 h on day 3; additional blood samples were obtained 30 min and 90 min after each meal, which were given at 0700 h, 1300 h, and 1900 h. Each meal contained one-third of the participant's estimated energy requirement [25]. After the evening meal was consumed

on day 2, participants fasted until the end of the hyperinsulinemic-euglycemic clamp procedure conducted on day 3. At 0700 h on day 3, a primed (8.0 μmol/kg) continuous (0.08 μmol/kg/min) intravenous infusion of [U^{13}-C]glucose (Cambridge Isotope Laboratories Inc., Andover, MA) was started and maintained for 210 min (basal period). An additional catheter was inserted into a radial artery to obtain arterial blood samples. At the end of the basal period, the infusion of [U-^{13}C]glucose was stopped and an insulin infusion was initiated at a rate of 50 mU/m^2/min (initiated with a two-step priming dose of 200 mU/m^2 BSA/min for 5 min followed by 100 mU/m^2/min for 5 min) for an additional 210 min. Euglycemia (~100 mg/dL) was maintained by variable rate infusion of 20% dextrose enriched to ~1% with [U-^{13}C]glucose. Blood samples were obtained before beginning the tracer infusion and every 6–7 minutes during the final 20 minutes of the basal and insulin infusion periods. Due to technical issues, one participant in the Diet+EX group did not complete the 24-h blood sampling after weight loss.

Skeletal muscle biopsy.

Skeletal muscle tissue was obtained during the basal period of the clamp procedure before and after the intervention in the Diet-ONLY (n=6) and Diet+EX (n=8) groups. Muscle tissue was obtained from the quadriceps femoris by using local anesthesia (lidocaine 2%) and a Tilley-Henkel forceps. Tissue samples were immediately rinsed in ice-cold saline and frozen in liquid nitrogen before being stored at −80 °C until processed for RNA sequencing.

Fecal collections

Fecal samples were collected at home, frozen at −20 °C and then transported to the CTRU in a polystyrene foam cooler containing frozen cold packs within 36-h of production. Samples were then stored at −80 °C freezer until processing for fecal 16S rRNA amplicon sequencing. During baseline (T0) and post-intervention (T3) testing, three fecal samples were collected ~7 days apart and the median of these replicates is reported. The T3 samples were collected when participants were weight stable for 1–4 weeks (Extended Data Fig. 2). Additional fecal samples were collected during progressive weight loss after approximately one (T1) and two (T2) months on the calorie-restricted diet. Baseline samples at T0 were collected while participants consumed their habitual diet and samples collected at T1, T2, and T3 were collected while the participants consumed the intervention diet.

Diet and exercise interventions

After completing baseline testing, subjects participated in a supervised weight loss program involving weekly individual dietary and behavioral education sessions with all food provided as packed-out meals. All subjects consumed a plant-forward “Pritikin type” diet (low in fat, sodium, and refined carbohydrates, and high in complex carbohydrates, primarily derived from plant-based sources [vegetables, fruit, whole grains, seeds, and legumes]), providing ~70% of energy as carbohydrates, ~15% as fats, and ~15% as protein. This specific diet was selected because it has demonstrated considerable beneficial effects on glycemic control and other cardiometabolic abnormalities [22,24]. The initial daily energy content of the diet provided 75% of estimated energy requirements [25]; subsequent energy intake was adjusted weekly as needed to achieve a 0.5%−1% weight loss per week until ~10% weight loss was achieved.

Participants in the Diet+EX group also performed six 1-hour exercise training sessions per week throughout the weight loss intervention; four sessions per week were conducted under the supervision of an exercise physiologist and two sessions were performed at home. Exercise sessions were performed at the time of day most convenient for each participant, which was usually in the afternoon or early evening. The prescribed exercise training was designed in accordance with the American College of Sports Medicine (ACSM) guidelines on physical activity and weight loss [51]. Supervised exercise sessions consisted of twice weekly sessions of aerobic exercise, one session of high-intensity interval training (HIIT), and one session of resistance training. During aerobic exercise sessions, participants were free to choose between exercising on treadmills, stationary cycle ergometers, or elliptical trainers at 65–75% of heart rate reserve (HRR). HIIT training sessions were comprised of 10 bouts of alternating 1 min “all-out” efforts and 1 min rest; and the balance of time was used to perform light aerobic exercise (<50% HRR). Resistance exercise sessions comprised of the following exercises: leg press, knee flexion, chest press, and seated row performed on a multi-station strength training equipment. Resistance training consisted of 4-week phases that alternated between high-intensity, low-volume (4 sets of 5–6 repetitions) and low-intensity, high-volume sessions (4 sets of 10–12 repetitions) sessions. Participants were also encouraged to perform two additional aerobic exercise sessions at home, and were given heart rate monitors to help ensure intensity targets were achieved.

Post-intervention testing

Once the targeted weight loss goal was achieved, energy intake was modified to maintain a stable body weight for 3–4 weeks (1.2 ± 0.3 and 1.1 ± 0.1% change in body weight in the Diet-ONLY and Diet+EX groups, respectively) before the testing procedures performed at baseline were repeated. Participants continued to consume packed-out meals throughout final testing and during the inpatient visit. Participants in the Diet+EX group continued the exercise intervention throughout the post-intervention testing with the final exercise sessions performed between 4 and 5 pm on the day of admission for the inpatient metabolic testing visit and from 4 to 5 pm the following day during the 24-h blood sampling protocol; these sessions involved 60 minutes of treadmill exercise at 70–80% HRR.

Sample analyses and bioinformatics

Members of the study team who conducted the sample analyses were blinded to participant group assignment.

Plasma substrate, insulin, C-peptide and cytokine concentrations.—Plasma glucose concentration was determined by using an automated glucose analyzer (Yellow Spring Instruments Co, Yellow Springs, OH). Plasma insulin, C-peptide, hemoglobin A1c (HbA1c), and lipid profile were determined by using an automated chemistry analyzer (Cobas 6000, Roche Diagnostics, Indianapolis, IN) in the Washington University Core Laboratory for Clinical Studies. Plasma cytokine concentrations were determined by using magnetic bead suspension assays and a Luminex 200 analyzer (Luminex Corp., Austin, TX). Plasma BCAA concentrations were determined by gas chromatography/mass spectrometry (GC-MS) using the EZ:faast Amino Acid Analysis kit (Phenomenex, Torrance, CA, USA). Plasma nonesterified fatty acid (NEFA) concentrations were determined by using

a colorimetric assay (FujiFilm, Osaka, Japan). Plasma glucose tracer-to-tracee ratio (TTR) was determined by using GC-MS and heptafluorobutyric (HFB) anhydride to form an HFB derivative of glucose [52].

Skeletal muscle RNA sequencing.—Total RNA was isolated from frozen samples of skeletal muscle by using QIAzol and an RNeasy mini kit in combination with an RNase-free DNase Set (Qiagen, Valencia, CA) [53]. One sample from the Diet+EX group was excluded because of poor RNA quality. Library preparation of the samples was performed with mRNA reverse transcribed to yield cDNA fragments, which were then sequenced by using an Illumina NovaSeq 6000 at the UC San Diego IGM Genomics Center. Expression of individual genes are presented as $\log_2$-transformed counts per million reads.

Plasma proteomics.—Plasma proteomic profiles were determined by using the SOMAscan Assay v4.0 (SomaLogic, Inc. Boulder, CO), which measures relative abundance of circulating proteins using a process that transforms protein concentrations into a corresponding signature of DNA aptamers, which is quantified on a DNA microarray [54]. Standard samples and negative controls are included with each assay, the resulting raw intensities are normalized and calibrated to control for inter-plate differences. This analysis identified 4,684 unique proteins that were used to compare the effect of weight loss in the Diet-ONLY and Diet+EX groups.

Fecal 16S amplicon sequencing.—DNA extraction from fecal samples, amplification of the V4 region of the 16S rRNA gene, and Illumina amplicon sequencing were performed at the University of California San Diego Center for Microbiome Innovation, according to the Earth Microbiome Project protocols [55]. Sequence data were processed by using QIIME2 (v2019.10) [56]. Paired-end sequences were processed and denoised using the q2-dada2 plugin. The phylogenetic tree was constructed by using fragment insertion by SEPP using the q2-fragment-insertion plugin [57]. Taxonomic assignment of amplicon sequence variants (ASVs) was performed by using a pre-trained Naïve Bayes classifier trained on the GreenGenes 13_8 database. Alpha diversity was quantified using Chao richness and Shannon entropy on count tables rarefied to 10,000 reads.

Calculations

Plasma concentration area under the curves (AUC) were calculated by using the trapezoidal method [58]. Insulin secretion rates (ISR) during the OGTT and 24-h study were calculated by using a stepwise ISR function to fit C-peptide concentrations to a 2-compartment model of C-peptide kinetics (SAAM II version 1.2.1, The Epsilon Group, Charlottesville, VA) using population-based C-peptide model parameters [59]. Whole-body insulin clearance rate (ICR; i.e., the volume of plasma cleared of insulin per minute) over a given time interval during the OGTT and 24-h study was calculated by dividing the ISR by the plasma insulin concentration after adjusting for insulin that accumulated and did not clear from plasma: $ICR_{xy} = [AUC\ ISR_{xy} - V \times (I_y - I_x)]/AUC\ Ins_{xy}$, where subscripts x and y denote time points for the interval, ISR_{xy} is the ISR over the time interval, V is the distribution volume for insulin (assumed to be 55 mL/kg FFM), and I_x and I_y are the plasma insulin concentrations at time points x and y [60]. The homeostasis model assessment

of insulin resistance (HOMA-IR) was calculated by dividing the product of the plasma concentrations of insulin (in μU/mL) and glucose (in mmol/L) by 22.5 [61]. The Matsuda insulin sensitivity index was calculated based on plasma glucose (in mg/dL) and insulin (in μU/mL) concentrations measured before and at 30, 60, 90 and 120 minutes after 75 g glucose ingestion [27]. The hepatic insulin sensitivity index (HISI) was calculated as the inverse of the product of plasma insulin concentration (in μU/mL) and endogenous glucose rate of appearance (Ra) into the systemic circulation per kg FFM. Glucose Ra was determined by dividing the glucose tracer infusion rate by the average plasma glucose TTR during the last 20 minutes of the basal period of the clamp procedure [26]. Insulin-stimulated glucose rate of disposal (Rd) was assumed to be equal to the sum of the endogenous glucose rate of appearance into the bloodstream and the rate of infused glucose during the last 20 minutes of the clamp procedure [26]. Whole-body insulin sensitivity was calculated as glucose Rd per kilogram FFM divided by the average plasma insulin concentration in pmol/L (glucose Rd/I) during the final 20 minutes of the clamp procedure, which primarily represents skeletal muscle insulin sensitivity [21]. β-cell function was assessed as the change in ISR in relation to the change in plasma glucose concentration during the first 30 min of the OGTT, when plasma glucose concentration rapidly increases [62].

Data collection and management

Study data were collected and managed by using REDCap version 7 electronic data capture tools hosted at Washington University School of Medicine.

Statistical analyses

Primary and secondary cardiometabolic outcomes.—The statistical significance of group differences in the intervention-induced changes in primary and secondary outcomes were analyzed by using two-tailed analysis of covariance (ANCOVA) with the baseline value as the covariate. In addition, 95% confidence intervals around the mean change within groups are provided to show the range of the change for each variable. Statistical analyses were performed by using Excel (Version 2016, Microsoft, Redmond, WA) and SPSS (version 28, IBM, Armonk, NY). No *P*-value corrections for multiple testing to control study-wide error rate were applied for secondary outomes.

Secondary outcomes related to potential mechanisms involved in regulating insulin sensitivity: muscle transcriptome, plasma proteome, and fecal microbiome.

RNA sequencing.: Differential expression analysis was performed comparing Before vs After weight loss in each group by using the edgeR R/Bioconductor package (version 3.40.2). Genes with $|\log_2 \text{fold change}| > 0.3$ and two-sided false discovery rate (FDR) < 0.05 were considered differentially expressed genes (DEGs). Functional enrichment analyses were performed on DEGs by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources 6.8 (http://david.ncifcrf.gov/) with Gene Ontology biological process (GO BP) Direct category. GO terms with two-sided FDR <0.01 were considered significantly enriched. To confirm the results of the DEG

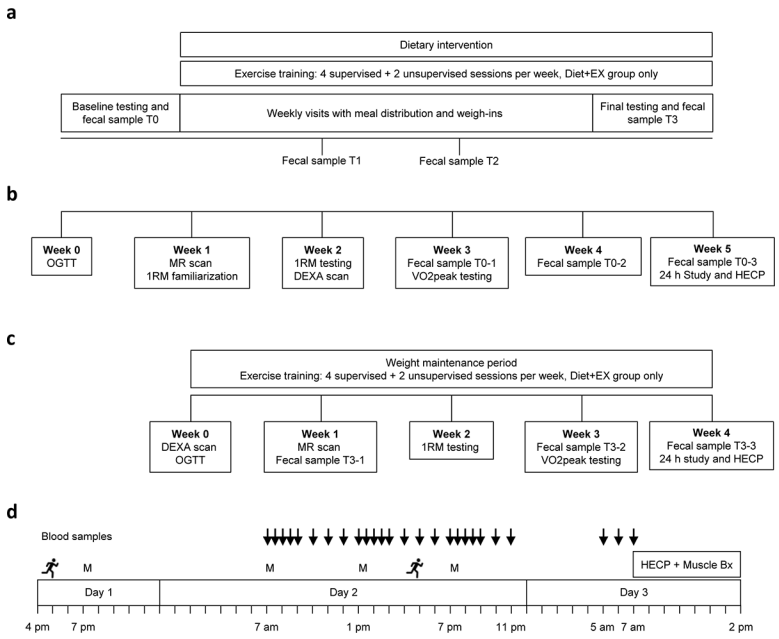
analysis, we identified key genes from the pathway analysis and analyzed these data by using ANCOVA.

SomaScan proteomics.: Statistical analyses were performed on the hybridization- and median-normalized, plate-scaled protein mean fluorescence intensity (MFI) data after $\log_2$ transformation and removal of proteins with a $\log_2$ MFI <7.5, leaving 4,684 unique proteins for analysis. Differential abundance of each protein from Before to After weight loss in each group was tested using *limma* (accounting for the mean-variance trend) [63]. *P*-values were adjusted using the Benjamini-Hochberg method and a *Padj* <0.20 was considered significant. Gene set enrichment analysis (GSEA) was conducted with the Gene Ontology biological process and cellular component categories (GO BP and GO CC, respectively) using the *t*-statistics from the *limma* procedure as input. SomaScan data were processed and analyzed in R using the SomaDataIO R package (version 5.1.0.9), and the *limma* (version 3.46.0), and fgsea (version 1.26.0) Bioconductor packages.

Gut Microbiome.: For the 16s microbiome data, all analyses were conducted: 1) within each group separately to determine group-specific changes during the interventions, and 2) given that virtually no differences between the groups were detected, a pooled analysis was performed to evaluate the effects of diet-induced weight loss on the gut microbiome. Alpha diversity metrics were analyzed using linear mixed effects models to account for the effect of subject-level variability. Differences in overall microbiome composition between groups and between timepoints within groups (Beta diversity) were assessed by using Permutational Multivariate Analysis of Variance using Distance Matrices (PERMANOVA) with the weighted UniFrac distance through Adonis using the following formula: study_group*study_period + sex. Adonis analysis was performed by using R (Version 4.1.3, The R Foundation for Statistical Computing, Vienna, Austria) and Bioconductor (Version 2.6.2) with the vegan package (Version 3.14). Differential abundance analysis was performed using a Bayesian linear mixed effects approach accounting for subject as a random effect. Study group, study period (T0-T3), their interaction, and sex were considered as fixed effects. Individual taxa were fit using a negative binomial model accounting for compositionality and overdispersion. For log-ratio analysis, the abundance sum of the 10 most associated ASVs was divided by the abundance sum of the 10 least associated ASVs (plus pseudocount of 1) in each sample.

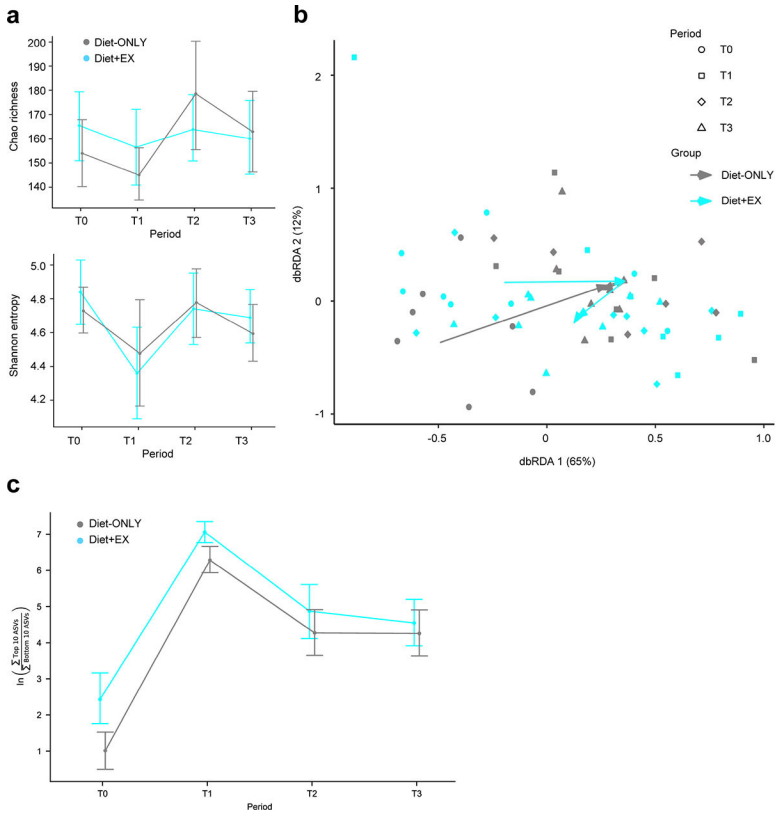
Power analysis.: We estimated that 8 participants/group would be needed to detect a 50% difference in the increase in whole-body (muscle) insulin sensitivity between groups, with a power of 0.9, a two-tailed test, and an α-value of 0.05. This power calculation was based on the following assumptions: i) the variability in muscle insulin sensitivity would be ~10 nmol/kg FFM/min per pmol/L of insulin, which is the variability we have observed previously in a similar study population [64]; and ii) weight loss in the Diet-ONLY group would double muscle insulin sensitivity (estimated to increase from 30 nmol/kg FFM/min per pmol/L of insulin to 60 nmol/kg FFM/min per pmol/L of insulin [2]).

Extended Data



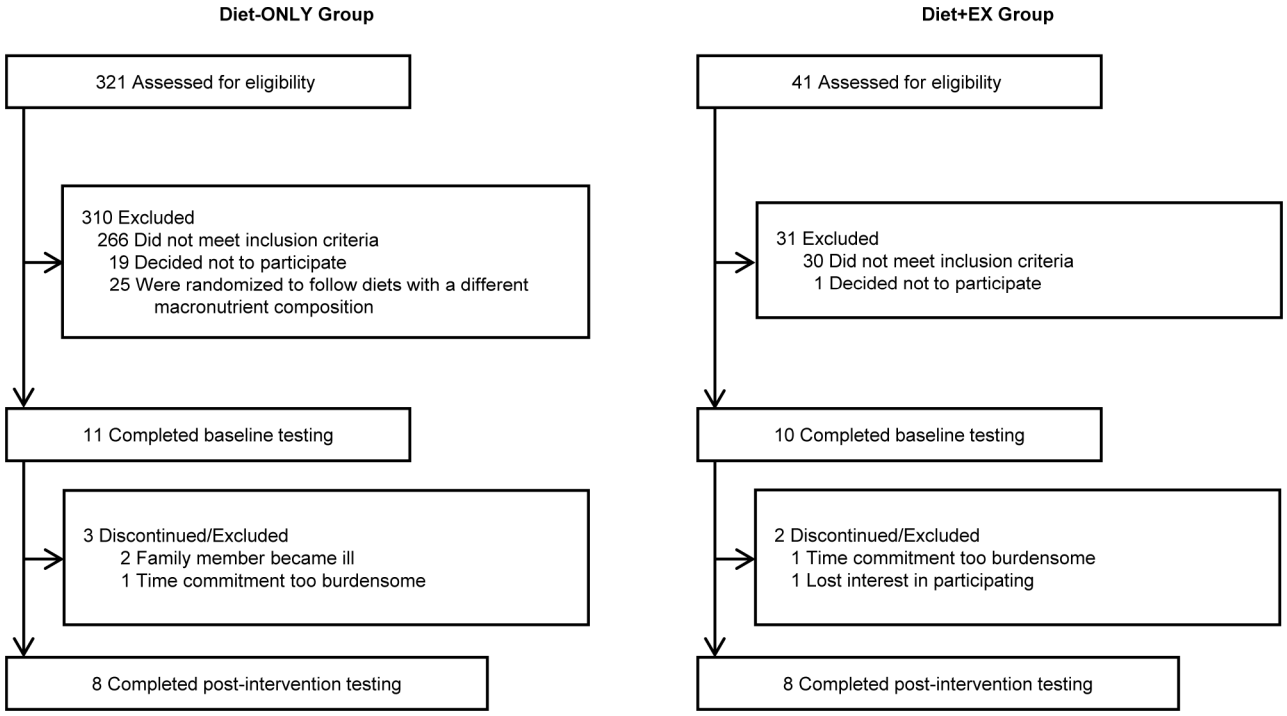
Extended Data Fig. 1.

Schematic diagrams of overall study protocol (a), schedule of individual assessments before (b) and after (c) the intervention and the protocol for inpatient 24-h study blood sampling schedule and hyperinsulinemic-euglycemic clamp procedure (HECP) plus muscle biopsy (Bx) (d). Blood sample timepoints are indicated by arrows and running figure indicates time of a 60-min bout of exercise performed in the Diet+EX group after the intervention. M=meal.



Extended Data Fig. 2. Effect of each intervention on the gut microbiome.

Measures of alpha diversity using both Chao richness (top) and Shannon entropy (bottom), before (T0) and at ~1 month (T1, 5.2 ± 0.7% weight loss), ~2 months (T2, 8.6 ± 0.8% weight loss), and ~4–5 months (T3, 10.5 ± 2.7% weight loss) after starting the intervention in the Diet-ONLY (n=7) and Diet+EX (n=8) groups. Data are means ± SEM (a). Beta diversity using Distanced-based Redundancy Analysis of the weight UniFrac distance, conditional on the between-person variability (b). Log-ratio analysis of 10 ASVs that increased the most and the 10 ASVs that decreased the most from T0 to T1 with treatment groups combined shown over time when stratified by study group. Data are log-ratio means ± SEM (c).



Extended Data Fig. 3.
Flow of study participants.

Extended Data Table 1.

Strength and cardiovascular fitness

	Diet-ONLY (*n*=6–7)			Diet+EX (*n*=8)			Between Groups
	Before	**After**	**Difference (95% CI)**	**Before**	**After**	**Difference (95% CI)**	***P-value***
Leg press (1-RM, kg)[a]	116 ± 24	123 ± 34	7 (−26, 39)	82 ± 7	94 ± 8	13 (2, 23)	0.171
Knee flexion (1-RM, kg)[a]	89 ± 10	85 ± 10	−4 (−11, 2)	65 ± 10	74 ± 9	9 (5, 13)	0.004
Seated row (1-RM, kg)[a]	77 ± 10	75 ± 9	−2 (−3, 0)	69 ± 8	78 ± 10	9 (−1, 18)	0.030
Chest press (1-RM, kg)[a]	69 ± 15	60 ± 12	−8 (−18, 1)	51 ± 7	56 ± 7	4 (−3, 12)	0.068
VO_{2peak} (mL/kg FFM/min)[b]	48.6 ± 4.5	47.2 ± 4.2	−1.4 (−2.7, 0)	48.2 ± 4.9	54.6 ± 6.2	6.4 (15, 11.3)	0.005
VO_{2peak} (mL/kg/min)[b]	27.2 ± 3.3	28.7 ± 3.5	1.5 (0.7, 2.4)	25.2 ± 2.5	31.2 ± 3.7	6.0 (2.7, 9.4)	0.004

Values before and after weight loss are expressed as means ± SEM. Abbreviations: 1-RM=one repetition maximum; FFM=fat-free mass; VO_{2peak}=peak oxygen consumption measured by using indirect calorimetry during incremental treadmill exercise to volitional exhaustion. Change values are expressed as mean and 95% confidence bounds within each group.

[a] Diet-ONLY group *n*=6.

[b] Diet-ONLY group n=7.

[c] Two-sided analysis of covariance without correction for multiple comparisons was used to determine between group differences after adjusting for values before weight loss.

Extended Data Table 2.

Fasting plasma markers of inflammation

	Diet-ONLY (n=8)			Diet+EX (n=8)			Between Groups
	Before	**After**	**Difference (95% CI)**	**Before**	**After**	**Difference (95% CI)**	***P-value***
PAI-1 (ng/mL)	63.8 ± 18.5	28.4 ± 6.0	−35.4 (−80.5, 9.8)	34.5 ± 8.2	15.9 ± 3.9	−18.6 (−34.0, −3.1)	0.640
IFN-γ (pg/mL)	15.2 ± 3.2	15.6 ± 3.4	0.4 (−1.3, 2.1)	11.4 ± 1.7	10.7 ± 1.9	−0.7 (−2.3, 0.8)	0.404
IL-1β (pg/mL)	0.75 ± 0.09	0.76 ± 0.09	0.01 (−0.21,0.23)	0.69 ± 0.09	0.69 ± 0.11	−0.00 (−0.08, 0.08)	0.853
IL-6 (pg/mL)	18.6 ± 9.9	12.9 ± 8.0	−5.7 (−15.7, 4.3)	16.1 ± 10.1	12.1 ± 7.8	−4.0 (−9.7, 1.7)	0.550
IL-10 (pg/mL)	15.6 ± 3.8	13.9 ± 3.6	−1.7 (−5.9, 2.4)	18.9 ± 7.5	16.1 ± 6.3	−2.8 (−7.1, 1.4)	0.459
IL-17 (pg/mL)	5.9 ± 1.9	6.2 ± 1.9	0.3 (−0.8, 1.5)	3.4 ± 0.9	3.0 ± 0.9	−0.4 (−1.2, 0.4)	0.214
MCP-1 (pg/mL)	8.8 ± 0.4	8.2 ± 0.5	−0.6 (−1.1, 0.0)	8.6 ± 0.6	8.1 ± 0.6	−0.5 (−1.2, 0.2)	0.879
TNF-α (pg/mL)	4.8 ± 0.4	5.0 ± 0.4	0.2 (−0.4, 0.7)	5.0 ± 0.5	4.7 ± 0.6	−0.3 (−1.1, 0.4)	0.191
CRP (mg/L)	5.7 ± 1.2	5.3 ± 1.2	−0.4 (−1.3, 0.5)	5.4 ± 1.2	4.5 ± 1.1	−0.9 (−3.3, 1.6)	0.615

Values before and after weight loss are expressed as means ± SEM. Change values are expressed as mean and 95% confidence bounds within each group. Two-sided analysis of covariance without correction for multiple comparisons was used to determine between group differences after adjusting for values before weight loss.

Extended Data Table 3.

Metabolic outcomes before and after intervention

	Diet-ONLY (n=8)			Diet+EX (n=8)			Difference between Groups
	Before	**After**	**Difference (95% CI)**	**Before**	**After**	**Difference (95% CI)**	***P*-value**
3-h oral glucose tolerance test [a]							
Glucose AUC (mg/dL × 3h)	476 ± 27	418 ± 11	−59 (−118, 1)	451 ± 8	404 ± 17	−47 (−77,−17)	0.790
Insulin AUC ([pmol/L × 3h] × 10^3)	2.91 ± 0.39	2.07 ± 0.20	−0.83 (−1.54, −0.13)	2.65 ± 0.44	1.37 ± 0.18	−1.29 (−2.10, −0.48)	0.008
ISR AUC ([pmol/min × 3h] × 10^3)	4.52 ± 0.52	3.64 ± 0.40	−0.89 (−1.51,−0.26)	3.61 ± 0.31	2.88 ± 0.16	−0.73 (−1.29, −0.18)	0.394
ICR AUC (L/min × 3h)	5.42 ± 0.38	5.80 ± 0.37	0.38 (−0.45, 1.22)	5.14 ± 0.69	7.49 ± 1.00	2.35 (0.31,4.39)	0.082
24-h blood sampling study [b]							

	Diet-ONLY (*n*=8)			Diet+EX (*n*=8)			Difference between Groups
	Before	**After**	**Difference (95% CI)**	**Before**	**After**	**Difference (95% CI)**	***P*-value**
Glucose AUC ([mg/dL × 24h] × 10^3)	2.66 ± 0.09	2.45 ± 0.06	−0.22 (−0.37, −0.06)	2.59 ± 0.03	2.36 ± 0.03	−0.23 (−0.35, −0.11)	0.334
Insulin AUC ([pmol/L × 24h] × 10^3)	11.3 ± 1.3	9.0 ± 1.5	−2.4 (−4.1,−0.6)	13.2 ± 3.0	6.0 ± 0.8	−7.2 (−13.5, −0.9)	0.030
ISR AUC ([pmol/min × 24h] × 10^3)	19.7 ± 2.1	15.3 ± 2.3	−4.5 (−7.9, −1.0)	17.3 ± 2.2	12.2 ± 1.1	−5.1 (−8.6, −1.6)	0.460
ICR AUC (L/min × 24h)	47.6 ± 2.6	51.4 ± 6.5	3.8 (−7.4, 14.9)	42.1 ± 4.6	61.4 ± 5.8	19.2 (12.9, 25.6)	0.009
NEFAAUC (mmol/L × 24h)	7.50 ± 0.70	5.92 ± 0.46	−1.57 (−2.95, −0.20)	6.27 ± 0.54	8.08 ± 0.39	1.81 (0.00, 3.61)	0.003
Insulin sensitivity							
Whole-body (muscle) insulin sensitivity	32.7 ± 3.3	48.4 ± 7.2	15.7 (4.7, 26.7)	38.4 ± 6.7	80.6 ± 7.6	42.2 (27.6, 56.8)	0.006
Hepatic insulin sensitivity Índex	2.72 ± 0.43	5.11 ± 0.89	2.39 (1.27, 3.51)	2.92 ± 0.33	7.67 ± 0.94	4.75 (2.95, 6.55)	0.014
Matsuda index[a]	1.39 ± 0.20	1.83 ± 0.16	0.44 (0.00, 0.88)	1.63 ± 0.44	2.91 ± 0.38	1.27 (0.18, 2.36)	0.037

Values before and after weight loss are expressed as means ± SEM. Abbreviations: ICR=insulin clearance rate; ISR=insulin secretion rate; NEFA=non-esterified fatty acids. Change values are expressed as mean and 95% confidence bounds within each group.

[a] Diet-ONLY group n=7.

[b] Diet+EX group n=7.

Whole-body insulin sensitivity represents glucose disposal rate from plasma per kilogram fat-free mass (FFM) divided by plasma insulin concentration during the hyperinsulinemic-euglycemic clamp procedure, which primarily represents skeletal muscle insulin sensitivity. Hepatic insulin sensitivity index was calculated as the reciprocal of the product of endogenous glucose production rate per kilogram FFM and plasma insulin concentration during basal conditions. The Matsuda insulin sensitivity index was calculated as 10,000 divided by the square root of the product of plasma glucose and insulin concentrations during the basal State and mean plasma glucose and insulin concentrations at 30, 60, 90 and 120 min during the oral glucose tolerance test. Two-sided analysis of covariance without correction for multiple comparisons was used to determine between group differences after adjusting for values before weight loss.

Extended Data Table 4.

Expression of key genes related to mitochondrial biogenesis, energy metabolism and angiogenesis in skeletal muscle.

	Diet-ONLY (*n*=6)			Diet+EX (*n*=7)			Dlfference between Groups
	Before	**After**	**Dlfference (95% CI)**	**Before**	**After**	**Dlfference (95% CI)**	***P*-value**
PPARGC1A	6.04 ± 0.18	6.16 ± 0.17	0.12 (−0.28, 0.52)	6.07 ± 0.12	6.84 ± 0.10	0.77 (0.57, 0.97)	0.001
MFF	5.06 ± 0.03	4.96 ± 0.09	−0.10 (−0.34, 0.14)	4.97 ± 0.06	5.26 ± 0.03	0.29 (0.17, 0.40)	0.009

	Diet-ONLY (*n*=6)			Diet+EX (*n*=7)			Dlfference between Groups
	Before	**After**	**Dlfference (95% CI)**	**Before**	**After**	**Dlfference (95% CI)**	***P*-value**
CS	8.00 ± 0.09	8.04 ± 0.06	0.04 (−0.06, 0.13)	7.97 ± 0.04	8.37 ± 0.06	0.40 (0.24, 0.55)	<0.001
MDH1	7.26 ± 0.12	7.24 ± 0.17	−0.02 (−0.32, 0.29)	7.15 ± 0.08	7.80 ± 0.15	0.65 (0.33, 0.98)	0.007
CYCS	6.33 ± 0.13	6.36 ± 0.17	0.03 (−0.35, 0.42)	6.43 ± 0.09	7.11 ± 0.11	0.67 (0.45, 0.90)	0.003
ATP5F1A	9.05 ± 0.10	9.02 ± 0.12	−0.03 (−0.24, 0.18)	8.81 ± 0.09	9.27 ± 0.10	0.47 (0.19, 0.74)	0.030
ACADVL	8.96 ± 0.04	8.72 ± 0.10	−0.24 (−0.51, 0.02)	8.66 ± 0.08	9.04 ± 0.12	0.38 (0.14, 0.62)	0.024
ACADM	6.85 ± 0.13	6.54 ± 0.21	−0.31 (−0.68, 0.06)	6.87 ± 0.15	7.21 ± 0.14	0.34 (0.22, 0.46)	0.001
LPL	5.20 ± 0.23	5.26 ± 0.18	0.06 (−0.32, 0.44)	5.28 ± 0.18	6.38 ± 0.23	1.11 (0.31, 1.91)	0.006
CD36	8.62 ± 0.12	8.38 ± 0.11	−0.23 (−0.52, 0.05)	8.28 ± 0.12	8.91 ± 0.18	0.63 (0.38, 0.89)	0.001
VEGFA	6.63 ± 0.14	6.48 ± 0.18	−0.15 (−0.69, 0.38)	6.59 ± 0.08	7.26 ± 0.13	0.67 (0.42, 0.92)	0.004
KDR	3.38 ± 0.13	3.20 ± 0.06	−0.18 (−0.52, 0.16)	3.19 ± 0.09	4.08 ± 0.12	0.89 (0.58, 1.19)	<0.001

Values before and after weight loss are $\log_2$CPM and expressed as means ± SEM. Change values are expressed as mean and 95% confidence bounds within each group. Two-sided analysis of covariance without correction for multiple comparisons was used to determine between group differences after adjusting for values before weight loss.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Source data for Figures 1, 2, 3, 4, 5c, and 6 and Extended Data Tables 1, 2, 3 and 4 are provided with this paper. The RNA-seq data generated during this study is available at the NCBI GEO database (accession no. GSE230002). We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources 6.8 to analyze the muscle RNA-seq data and it is available at http://david.ncifcrf.gov/. The 16S data has been uploaded through EBI https://www.ebi.ac.uk/ena under the study identifier PRJEB61649 and the GreenGenes 13_8 database was used for taxonomic assignment for the

ASVs detected in this study (https://data.qiime2.org/2022.11/common/gg-13-8-99-515-806-nb-classifier.qza).

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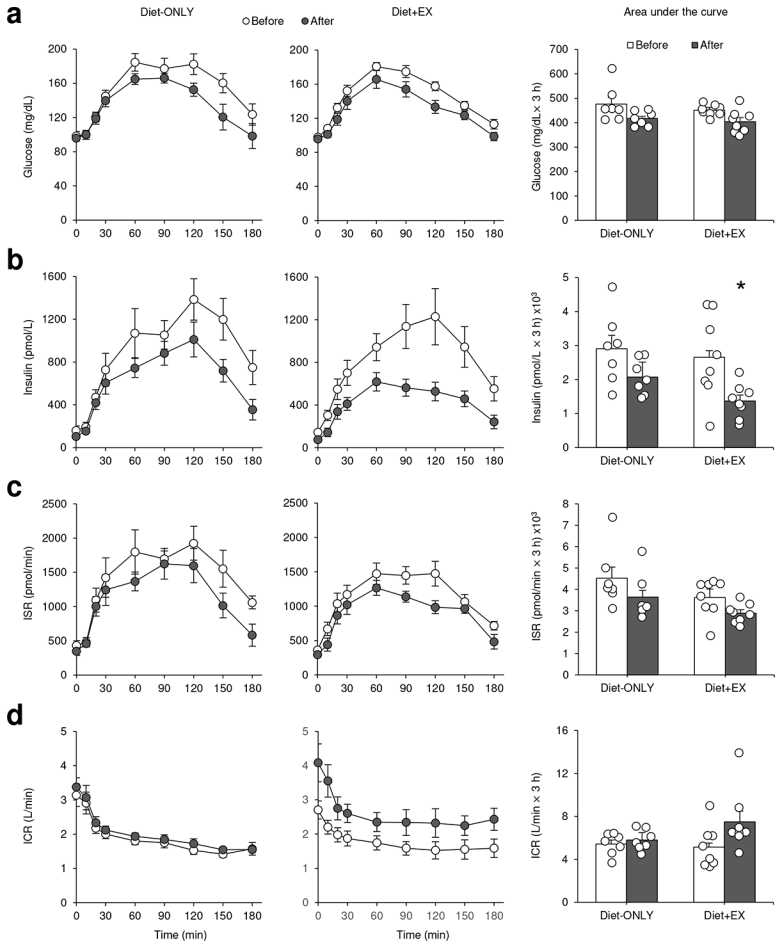

Fig. 1. Glucose and insulin responses to glucose ingestion.

Plasma concentrations and areas under the curve (AUC) for glucose (**a**), insulin (**b**), insulin secretion rate (ISR) (**c**) and insulin clearance rate (ICR) (**d**) in response to glucose ingestion in the Diet-ONLY (n=7) and Diet+EX (n=8) groups before (white circles and bars) and after ~10% weight loss (gray circles and bars). Data are means ± SEM. Analyses of covariance with baseline values as covariates were used to compare weight loss-induced changes in AUCs between groups. Two-sided ANCOVA without correction for multiple comparisons: *P=0.008 *vs.* Diet-ONLY value.

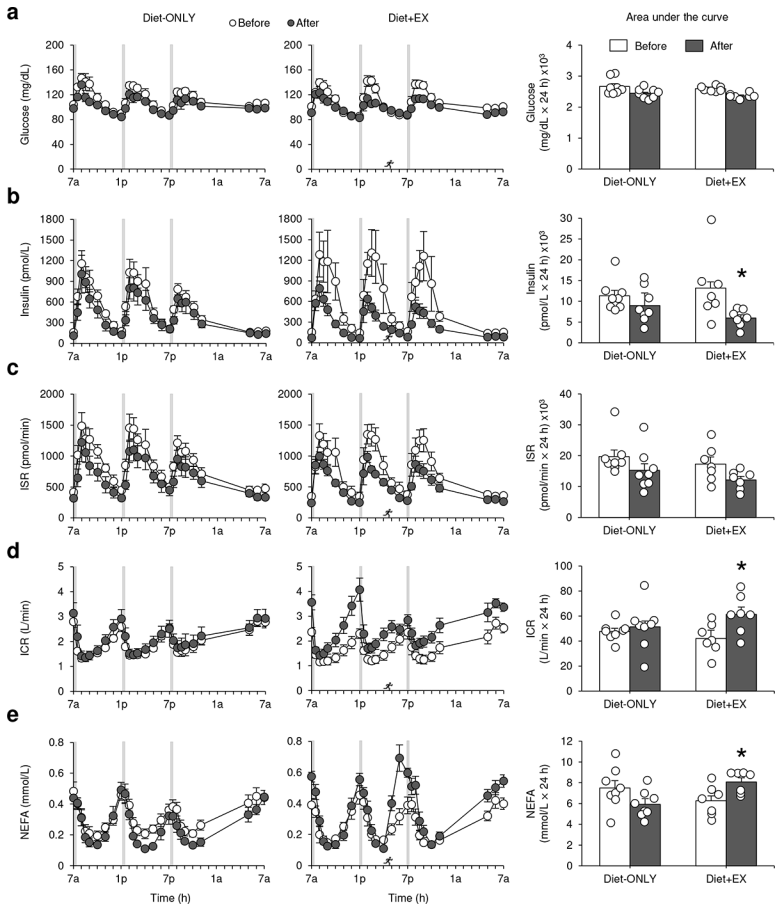

Fig. 2. 24-hour plasma glucose, insulin and nonesterified fatty acid dynamics.

Plasma concentrations and areas under the curve (AUC) for glucose (**a**), insulin (**b**), insulin secretion rate (ISR) (**c**), insulin clearance rate (ICR) (**d**), and nonesterified fatty acids (NEFA) (**e**) obtained over 24 hours in the Diet-ONLY (n=8) and Diet+EX (n=7) groups before (white circles and bars) and after ~10% weight loss (gray circles and bars). Gray vertical bars and running figure indicate time of mixed-meal ingestion and a 60-min bout of exercise performed in the Diet+EX group after ~10% weight loss, respectively. Detailed timeline is presented in Extended Data Fig. 1. Data are means ± SEM. Analyses of covariance with baseline values as covariates were used to compare weight loss-induced changes in AUCs between groups. Two-sided ANCOVA without correction for multiple comparisons: *P=0.030, 0.009 and 0.003 *vs.* Diet-ONLY value for plasma insulin, ICR and plasma NEFA AUCs, respectively.

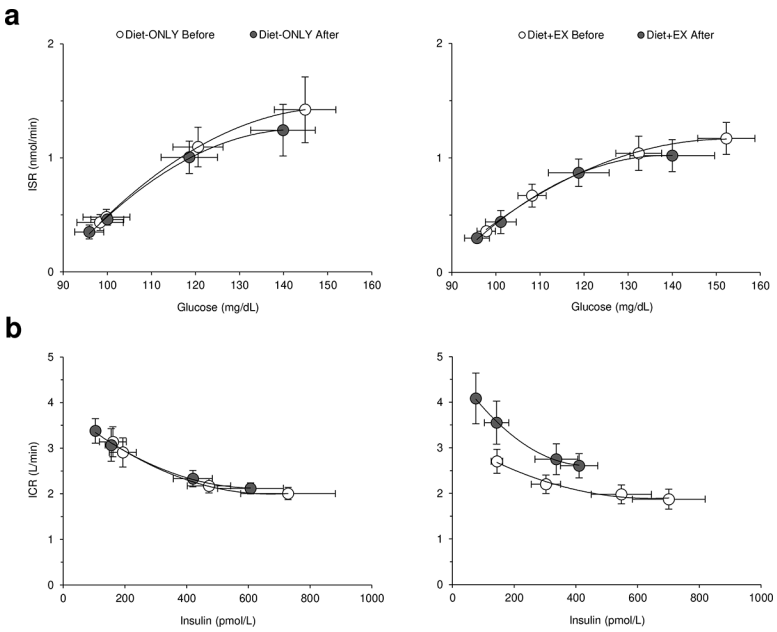

Fig. 3. β-cell function and insulin clearance during the first 30 minutes of the oral glucose tolerance test.

Relationships between plasma glucose concentration and insulin secretion rate (ISR) (**c**), and plasma insulin concentration and insulin clearance rate (ICR) (**b**) before and during the first 30 minutes after ingesting 75 g of glucose in the Diet-ONLY (*n*=7) and Diet+EX (*n*=8) groups before (white circles) and after ~10% weight loss (gray circles). Data are means ± SEM.

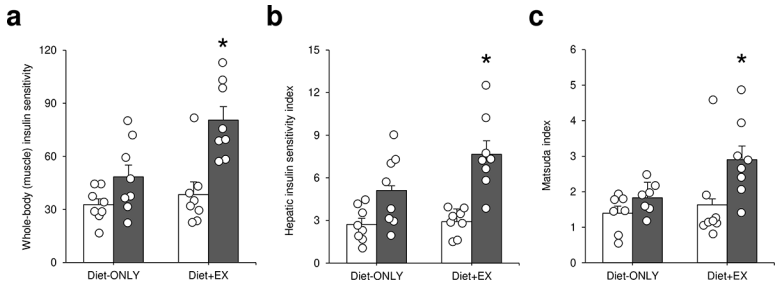

Fig. 4. Effect of Diet-ONLY and Diet+EX on insulin sensitivity.

Whole-body (primarily muscle) insulin sensitivity (glucose disposal rate per kilogram fat-free mass (FFM) divided by plasma insulin concentration during the hyperinsulinemic-euglycemic clamp procedure) (**a**), hepatic insulin sensitivity index (the reciprocal of the product of endogenous glucose production rate per kilogram FFM and plasma insulin concentration during basal conditions) (**b**), and the Matsuda insulin sensitivity index (10,000 divided by the square root of the product of plasma glucose and insulin concentrations during the basal state and mean plasma glucose and insulin concentrations at 30, 60, 90 and 120 min during the oral glucose tolerance test) (**c**) in the Diet-ONLY (n=8 in panels a and b, and n=7 in panel c) and Diet+EX (n=8 for all panels) groups before (white bars) and after ~10% weight loss (gray bars). Data are means ± SEM. Analyses of covariance with baseline values as covariates were used to compare weight loss-induced changes in AUCs between groups. Two-sided ANCOVA without correction for multiple comparisons: *P=0.006, 0.014 and 0.037 *vs.* Diet-ONLY value for whole-body insulin sensitivity, hepatic insulin sensitivity and Matsuda insulin sensitivity index, respectively.

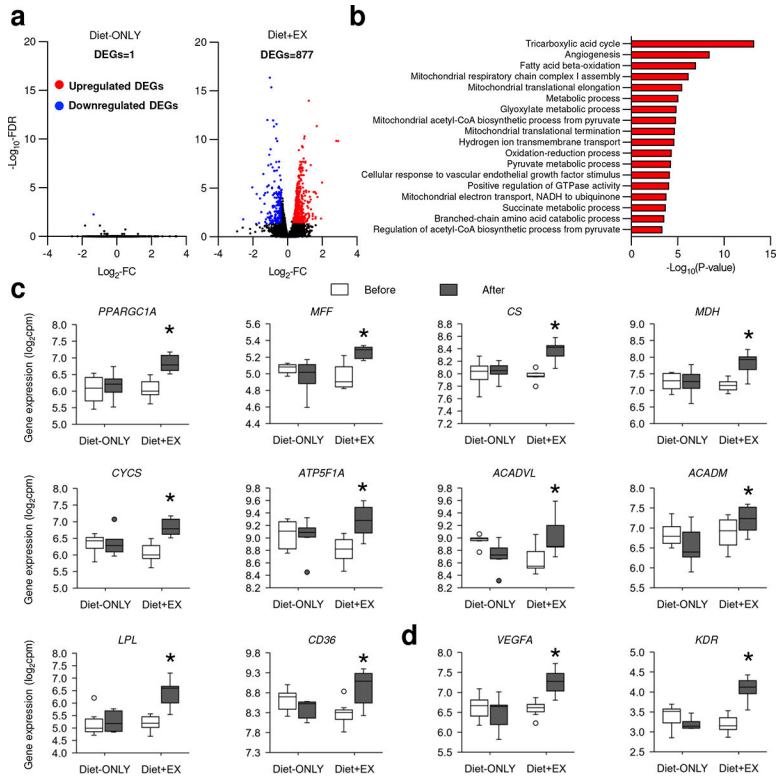

Fig. 5. Skeletal muscle RNA sequencing.

Skeletal muscle tissue obtained in the Diet-ONLY (*n*=6) and Diet+EX (*n*=7) groups before and after weight loss were evaluated by using RNA-sequencing (RNA-seq). Volcano plots of skeletal muscle RNA-seq data with $\log_2$-fold change (FC) (X-axis) and two-sided $-\log_{10}$false discovery rate (FDR) (Y-axis) (**a**). The number of differentially expressed genes (DEGs, $|\log_2 FC| > 0.3$ and two-sided FDR<0.05) between before and after weight loss in each group is shown. Biological pathways significantly (two-sided FDR<0.01) enriched with upregulated DEGs between before and after weight loss in the Diet+EX group (**b**). Expression of key genes that regulate mitochondrial biogenesis and energy metabolism, (**c**; peroxisome proliferator-activated receptor γ coactivator 1 α, *PPARGC1A*; mitochondrial fission factor, *MFF*; citrate synthase, *CS*; malate dehydrogenase 1, *MDH1*; cytochrome C somatic, *CYCS*; ATP Synthase F1 Subunit α, *ATP5F1A*; very long-chain and medium-chain specific acyl-CoA dehydrogenases, *ACADVL* and *ACADVM,* respectively; Lipoprotein lipase, *LPL*; and Cluster of differentiation 36, *CD36*) and expression of genes involved in regulating angiogenesis (**d**; vascular endothelial growth factor α, *VEGFA;* vascular endothelial growth factor receptor 2, *KDR*) in the Diet-ONLY and Diet+EX groups before (white bars) and after ~10% weight loss (gray bars). Data in panels C and D are median (central horizontal line), 25th and 75th percentiles (box) and minimum and maximum values (vertical lines) and outliers (circles), and were analyzed by using analyses of covariance with baseline values as covariates to compare weight loss-induced changes between groups. Two-sided ANCOVA without adjustment for multiple comparisons: **P*=0.001, 0.009, <0.001, 0.007, 0.003, 0.030, 0.024, 0.001, 0.006, 0.001, 0.004, <0.001 *vs.* Diet-ONLY value for *PPARGC1A*, *MFF*, *CS*, *MDH1*, *CYCS*, *ATP5F1A*, *ACADVL*, *ACADVM, LPL*, *CD36*, *VEGFA, and KDR* respectively.

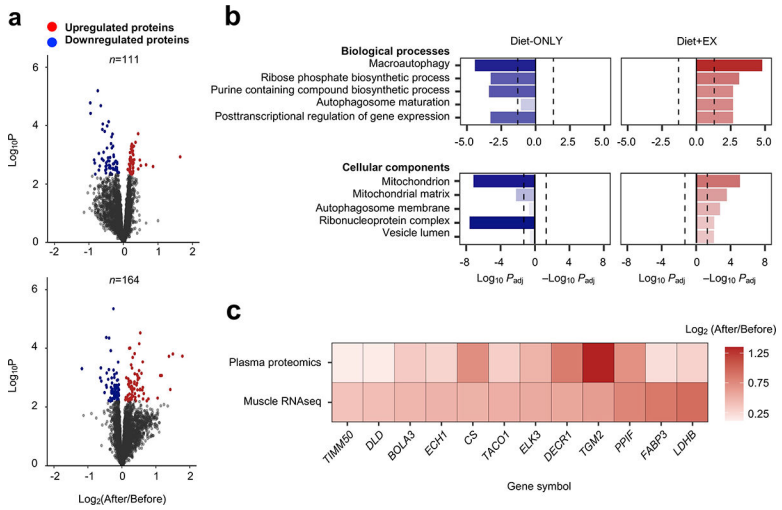

Fig. 6. Changes in the plasma proteome.

Volcano plots of proteins with differential abundance after weight loss in the Diet-ONLY (n=8) and Diet+EX groups (n=8) (**a**). Functional enrichment analyses were performed to identify biological pathways significantly enriched (two-sided *Padj*<0.20) with proteins with differential abundance after weight loss. The top 5 most significantly enriched pathways in the GO Biological process and Cellular component databases in the Diet+EX group and the corresponding changes in the Diet-ONLY group. Vertical dashed lines represent two-sided Log_{10}*Padj*<0.05 (**b**). Plasma proteins related to mitochondrial biology and energy metabolism (mitochondrial import inner membrane translocase subunit TIM50, *TIMM50*; mitochondrial dihydrolipoyl dehydrogenase, *DLD*; bolA-like protein 3, *BOLA3*; enoyl CoA hydratase 1, *ECH1*; citrate synthase, *CS*; translational activator of cytochrome c oxidase 1, *TACO1*; ETS domain-containing protein Elk-3, *ELK3*; 2,4-dienoyl-CoA reductase 1, *DECR1*; transglutaminase 2, *TGM2*; mitochondrial peptidyl-prolyl cis-trans isomerase F, *PPIF*; fatty acid binding protein 3, *FABP3*; lactate dehydrogenase B, *LDHB*) that significantly increased (*Padj*<0.20) after the intervention in conjunction with a significant (two-sided FDR *P*<0.05) increase in skeletal muscle expression of the encoding genes in the Diet+EX group (**c**). Differential abundance of each protein from Before to After weight loss in each group was tested by using empirical Bayes moderated paired t-tests and *P*-values were adjusted by using the Benjamini-Hochberg method and a two-sided *Padj* <0.20 was considered significant.

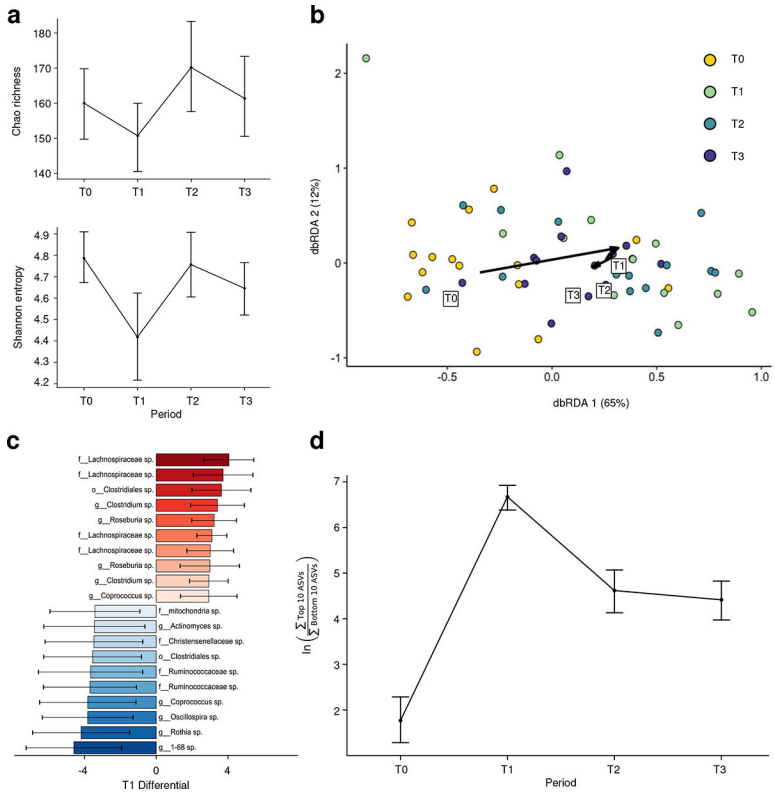

Fig. 7. Changes in gut microbiota.

Measures of alpha diversity using both Chao richness (top) and Shannon entropy (bottom), before (T0) and at ~1 month (T1, 5.2 ± 0.7% weight loss), ~2 months (T2, 8.6 ± 0.8% weight loss), and ~4–5 months (T3, 10.5 ± 2.7% weight loss) after starting the intervention all participants (all panels are n=15; Diet-ONLY n=7 and Diet+EX n=8). Data are means ± SEM (**a**). Beta diversity using Distanced-based Redundancy Analysis of the weight UniFrac distance, conditional on the between-person variability (**b**). Differential abundance results of the 10 ASVs that increased the most and the 10 ASVs that decreased the most from T0 to T1 with treatment groups combined. Red and Blue colored bars represent posterior mean values ± SD (**c**). Log-ratio analysis of top and bottom 10 ASVs shown in panel c over time with both groups combined. Data are log-ratio means ± SEM (**d**).

Table 1.

Participant characteristics before and after intervention

	Diet-ONLY (*n*=8)			Diet+EX (*n*=8)			Between Groups
	Before	**After**	**Difference (95% CI)**	**Before**	**After**	**Difference (95% CI)**	***P-value***
Body composition							
Body mass (kg)	114.8 ± 8.7	103.3 ± 8.7	−11.5 (−13.3, −9.7)	116.5 ± 10.6	104.2 ± 9.6	−12.3 (−16.3, −8.3)	0.682
BMI (kg/m^2)	39.1 ± 1.9	35.2 ± 2.1	−3.9 (−4.5, −3.4)	39.6 ± 2.2	35.4 ± 2.0	−4.2 (−5.3, −3.1)	0.676
Body fat (%)	45.6 ± 2.6	41.3 ± 3.2	−4.3 (−5.8, −2.8)	47.3 ± 1.2	42.9 ± 1.0	−4.4 (−5.5, −3.2)	0.981
Fat-free mass (kg)	61.1 ± 4.4	59.1 ± 4.4	−1.9 (−2.9, −1.0)	60.6 ± 5.4	59.1 ± 5.1	−1.5 (−2.6, −0.3)	0.502
Appendicular lean mass (kg)	27.8 ± 2.2	26.3 ± 2.1	−1.5 (−2.1, −0.9)	28.8 ± 3.2	27.6 ± 2.9	−1.2 (−2.1, −0.3)	0.387
IHTG content (%)[a]	19.5 ± 4.1	9.6 ± 3.0	−9.9 (−16.5, −3.3)	11.3 ± 2.6	6.9 ± 1.5	−4.5 (−7.9, −1.1)	0.540
Strength and cardiorespiratory fitness							
Sum of 1-RMs (kg)[b]	351 ± 52	343 ± 53	−8 (−38, 23)	267 ± 23	301 ± 26	35 (13, 56)	0.025
VO_{2peak} (L/min)[c]	3.0 ± 0.4	2.9 ± 0.4	−0.2 (−0.3, −0.1)	2.9 ± 0.4	3.3 ± 0.5	0.3 (0.0, 0.6)	0.007
Fasting blood chemistries							
Glucose (mg/dL)	98.0 ± 3.2	91.9 ± 2.2	−6.1 (−12.8, 0.6)	99.8 ± 3.9	90.0 ± 1.5	−9.8 (−17.0, −2.6)	0.113
Insulin (pmol/L)	186.7 ± 29.6	116.1 ± 21.6	−70.6 (−92.6, −48.5)	173.6 ± 25.7	79.0 ± 11.8	−94.5 (−133.2, −55.8)	0.009
HOMA-IR	7.7 ± 1.4	4.3 ± 0.8	−3.3 (−4.8, −1.8)	7.2 ± 1.2	3.0 ± 0.5	−4.2 (−6.1, −2.4)	0.001
HbA1c (%)	5.8 ± 0.3	5.3 ± 0.1	−0.5 (−1.0, 0.0)	5.7 ± 0.2	5.2 ± 0.1	−0.5 (−0.7, −0.3)	0.976
Triglyceride (mg/dL)	178 ± 38	116 ± 22	−62 (−116, −8)	140 ± 12	103 ± 10	−37 (−55, −18)	0.688
HDL-cholesterol (mg/dL)	35.0 ± 1.6	32.1 ± 1.5	−2.9 (−5.7, −0.1)	38.5 ± 2.7	37.3 ± 3.0	−1.3 (−4.9, 2.4)	0.343
LDL-cholesterol (mg/dL)	96 ± 7	94 ± 7	−2 (−20, 16)	116 ± 11	108 ± 9	−7 (−21,6)	0.839
Total cholesterol (mg/dL)	167 ± 11	150 ± 10	−17.3 (−39.9, 5.4)	182 ± 13	166 ± 10	−15.6 (−30.8, −0.4)	0.450
NEFA (mmol/L)	0.48 ± 0.06	0.44 ± 0.04	−0.04 (−0.16, 0.08)	0.42 ± 0.04	0.56 ± 0.03	0.15 (−0.01,0.31)	0.038
BCAAs (mmol/L)	764 ± 41	634 ± 47	−130 (−202, −58)	718 ± 32	623 ± 19	−94 (−161, −27)	0.630

Values before and after weight loss are expressed as means ± SEM. Change values are expressed as mean and 95% confidence bounds within each group. Abbreviations: 1-RM=one repetition maximum; BMI=body mass index; BCAA=branched-chain amino acids; HDL= high-density lipoprotein; HbA1c=hemoglobin A1c; HOMA-IR=homeostatic model assessment of insulin resistance; IHTG=intrahepatic triglyceride; LDL=low-density lipoprotein; NEFA=non-esterified fatty acids; Sum of 1-RMs=total weight lifted during leg press, knee flexion, seated row, and chest press one-repetition maximal strength tests.

[a]Diet+EX group *n*=7

[b] Diet-ONLY group $n=6$

[c] Diet-ONLY group $n=7$. Two-sided analysis of covariance without correction for multiple comparisons was used to determine between groups differences after adjusting for values before weight loss.